BIOREMEDIATION OF OIL CONTAMINATED WASTEWATER USING MIXED CULTURE

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"I hereby declare that I have read this thesis and in my opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)"

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A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

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MAY 2008

I declare that this thesis entitled "bioremediation of oil contaminated wastewater using mixed culture" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree

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Special Dedication to my family members that always love me,
My friends, my fellow colleague
and all faculty members

For all your Care, Support and Believe in me

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ABSTRACT

The objective of this research is to study the effect of the oil degradation based on the different temperature and different oil concentration using mixed culture from domestic wastewater. The evaluation is based on microorganism activities by the rate of oil degradation. The inoculum used was a mixed culture containing oil-degrading microorganism isolates from the sewage system located at Perak. For the wastewater used was artificially made by using palm oil as carbon substrate and medium for the microorganism to study the effect of temperature and oil concentration. Wastewater was treated by using the inoculum of the mixed culture for 20 days of incubation time and temperature range from 10^{o}C to 60^{o}C. From the experiment, it was observed that, the rate of oil degradation ~~is~~ was high at mesophilic condition which was at temperature of 30^{o}C resulted of 4.722 g from 10 g of oil have been degraded during the incubation time. The result also showed that, oil concentration at high value can limit the rate of oil degradation. It showed that rate of oil degradation was inversely proportional to the concentration of oil. From this study, it is shown that the effectiveness of oil degradation is increased by increasing in temperature and the optimum temperature in this study was 30^{o}C at low oil concentration.

ABSTRAK

Objektif kajian ini adalah untuk mengkaji perubahan dalam–penguraian minyak berdasarkan perbezaaan suhu dan perbezaan kepekatan minyak dengan menggunakan kultur campuran daripada air buangan domestik. Penilaian adalah berdasarkan kadar penguraian minyak daripada aktiviti mikroorganisma. Inokulum yang digunakan adalah kultur campuran yang mengandungi mikroorganisma pengurai minyak yang diasingkan daripada air buangan domestik yang terletak di Perak. Untuk air buangan yang digunakan, ia dihasilkan secara sintetik dengan menggunakan minyak sawit sebagai sumber karbon untuk mikroorganisma dalam mengkaji kesan perbezaan suhu dan perbezaan kepekatan minyak terhadap kadar penguraian minyak. Air buangan sintetik ini akan dirawat menggunakan inokulum daripada kultur campuran selama 20 hari pada suhu bermula dari $10^{o}C$ hingga $60^{o}C$. Daripada eksperimen ini didapati kadar penguraian minyak adalah tinggi dalam keadaan suhu yang *mesophilic* iaitu pada suhu $30^{o}C$ dengan penguraian minyak sebanyak 4.722 g daripada 10 g minyak sepanjang tempoh rawatan. Keputusan turut menunjukkan kepekatan minyak yang tinggi akan menghadkan penguraian minyak. Kadar penguraian minyak adalah berkadar songsang terhadap kepekatan minyak. Daripada kajian ini, didapati bahawa kebolehan mengurai minyak oleh kultur campuran akan meningkat sekiranya suhu rawatan semakin tinggi dan keadaan yang optimum untuk penguraian minyak bagi kajian ini adalah pada suhu $30^{o}C$ dan pada kepekatan minyak yang rendah.

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LIST OF SYMBOLS

%	-	Percentage
BOD	-	Biochemical oxygen demand
cm	-	Centimeter
C_{oil}	-	Oil concentration
g	-	Gram
h	-	Hour
L	-	Liter
min	-	Minute
ml	-	Milliliter
N	-	Nitrogen
ºC	-	Degree celcius
P	-	Phosphorus
PAHs	-	Polyaromatic hydrocarbons
PCBs	-	Polychlorinated biphenyls
rpm	-	Revolution per minute
TCE	-	Trichloroethylene
V_{sample}	-	Sample volume

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Bioremediation is defined as any process that uses microorganisms or their enzymes to destroy or reduce the concentrations of hazardous wastes from contaminated sites without further disruption to the local environment. It is a relatively slow process, requiring weeks to months to effect cleanup. If done properly, it can be very cost-effective. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and the environment. This is an attractive process due to its cost effectiveness and the benefit of pollutant mineralization to CO_2 and H_2O (Mills *et al.*, 2004). The microorganisms may be endogenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes (Margesin and Schinner, 2001).

Biodegradation of a compound is often a result of the actions of multiple organisms. During biodegradation, oil is used as an organic carbon source by a microbial process, resulting in the breakdown of oil components to low molecular weight compounds. In another words, biodegradation of oil contaminants can be described as the conversion of chemical compounds by microorganisms into energy, cell mass and biological products. The key component in bioremediation is the microorganisms, which produce the enzymes involved in the degradative reactions leading to the elimination or detoxification of the chemical pollutant (Rahman *et al.*, 2002). The goal of bioremediation is to degrade organic pollutant which is oil to concentrations below the limits established as safe or acceptable by regulatory agencies. Effective bioremediation requires nutrients to remain in contact with the oiled material and the concentrations should be sufficient to support the maximal growth rate of the oil-degrading bacteria throughout the cleanup operation. The success of oil wastewater bioremediation depends on our ability to establish those conditions in the contaminated environment (Reynolds *et al*., 1989).

1.2 Problem statement

Oil contaminated wastewater has posed a great hazard for environment and marine ecosystems. Oil is major component in domestic wastewater that causes severe environment pollution. It can form oil films on water surfaces, preventing the diffusion of oxygen from air into water and leading to the death of many forms of aquatic life. The traditional treatment of oil contaminated wastewater such as use of straw or plant material as an absorbent of oil, biosurfactants to cleanup oiled surfaces (Banat *et al*., 1991), oil-water separation and other methods. But, all of these physical and chemical methods can not degrade and remove the oil thoroughly (Ollis, 1992). Biological methods can be most effective in the removal of thin oil films spread on the surface of water, where physical or chemical methods are not effective. So far, bioremediation suggests an effective method.

During bioremediation, oil is used as an organic carbon source by a microbial process, resulting in the breakdown of oil components to low molecular weight compounds. This technology accelerates the naturally occurring biodegradation under optimized conditions such as oxygen supply, temperature, pH, the presence or addition of suitable microbial population and nutrients, water content and mixing. Like other technologies, bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, so it is not easy to predict the rates of clean up for a bioremediation exercise and there are no rules to predict if a contaminant can be degraded (Banat *et al*., 1991).

Bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. So, bioremediation methods have focused on the addition of microorganisms or nutrients concentration and the temperature dependant condition of the process environment. The main requirements for degradation of oil by microorganism are energy sources and carbon sources. Biostimulation is the addition of substrates, vitamins, oxygen and other compounds that stimulate microorganism activity, so that they can degrade the waste faster. The addition of materials to encourage microbiological biodegradation of oil which has received the most attention, notably after the "Exxon Valdez" incident (Swannel *et al*., 1996), however, such as low water temperature are not favorable for bioremediation.

The bioremediation treatment of oils contaminated wastewater under high temperature conditions is expected to be advantageous due to favorable changes in most physical properties of these hydrophobic compounds with increasing temperature (Thomas *et al*., 1987). The melting point of oil is often well above ambient temperatures. Above their melting temperature, these substances become more accessible to microorganisms and their enzymes. Both diffusion coefficients and the solubility of oil in aqueous media increase significantly with rising temperatures allowing for a better mass transfer (Thomas *et al*., 1987).

1.3 Objectives of study

The objectives of this study are as follows:

a) To study the effect different temperature on the rate oil degradation
b) To study rate of degradation based on different oil concentration in wastewater treatment

1.4 Scope of study

The scope of this study is to find out the different in the rate of oil degradation of oil contaminated wastewater using mixed culture origin from the sewage system located at Perak in different incubation temperature and different oil concentration.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioremediation

Bioremediation means to use biological organisms to solve an environmental problem such as contaminated soil or contaminated water. In other words it is a technology for removing pollutants from the environment thus restoring the original natural surroundings and preventing further pollution. Bioremediation may be employed in order to attack specific contaminants, such as chlorinated pesticides that are degraded by bacteria, or a more general approach may be taken, such as oil contaminated wastewater that are broken down using multiple techniques including the addition of biostimulation to facilitate the decomposition of oil by bacteria (Jorgensen *et al*., 1999). Oil may contaminate water well below the surface of the water, injecting the right organisms, in conjunction with oxygen-forming compounds, may significantly reduce concentrations after a period of time. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate (Ayotamuno *et al*., 2002).

Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result (Jorgensen *et al*., 1999). Generally, bioremediation technologies can be classified as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site while ex situ involves the removal of the contaminated material to be treated elsewhere (Sasikumar and Papinazath, 2003). Different techniques are employed depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. Ex situ techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation for soil or pumping for water (Vidali, 2001)

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2.2 In situ bioremediation

The bioremediation methods employed will depend on the area contaminated, the properties of the compounds involved, the concentration of the contaminants and the time required to complete the bioremediation. The in situ process includes bioventing, biosparging, biostimulation, bioaugmentation and phytoremediation (Vidali, 2001).

2.2.1 Bioventing

Bioventing is the most common in situ treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface (Chipasa and Medrzycka, 2006)

2.2.2 Biosparging

Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system (Vidali, 2001)

2.2.3 Bioaugmentation

Bioaugmentation is the addition of microorganisms that specifically degrade the oil at the site of the oil spill. The oil-degradation organisms were collected from other sites and commercially cultivated them. They are selected to withstand harsh environmental conditions such as high salt and variable temperature combined with a superior ability to use the resources such as oxygen, nitrogen, phosphorus and others sources available. They also able to out compete indigenous microorganisms, so they can clean up the site rapidly (Campo *et al.*, 2007). It is proposed by proponents of bioaugmentation, once the oil which is the carbon source or substrate is used up, these organisms have no advantage over the native microorganisms present so eventually they decrease in numbers and disappear. The increase in the efficiency of the system was the result of an increased concentration of bacterial cells, which was accompanied by increased microbial activity, growth and maintenance of microbial populations that were associated with attached growth systems (Chipasa and Medrzycka, 2006)

2.2.4 Biostimulation

Biostimulation is the addition of substrates, vitamins, oxygen and other compounds that stimulate microorganism activity so that they can degrade the waste faster. Biostimulation of microorganisms by the addition of nutrients because the input of large quantities of carbon sources tends to result in a rapid depletion of the available pools of major inorganic nutrients such as N and P (Sang-Hwan *et al.*, 2007). An example of this is the addition of fertilizer to an oil wastewater. This works by supplying nutrients that are limiting the growth of the bacteria for the oil contaminated wastewater such as nitrogen and phosphorous. With this addition, the organisms can rapidly degrade the oil utilizing it as the carbon source and the fertilizer as the nitrogen and phosphorous source (Campo *et al.*, 2007).

2.2.5 Phytoremediation

Vegetation based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. In natural ecosystems, plants act as filters and metabolize substances generated by nature. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water. Its potential for encouraging the biodegradation of organic contaminants requires further research, although it may be a promising area for the future (Truu *et al*., 2003)

2.3 Ex situ bioremediation

If the contaminated material is excavated it can be treated on or off site which is often a more rapid method of decontaminating the area. The techniques that can be used are include land farming, composting, biopiles and bioreactors (Vidali, 2001)

2.3.1 Land farming

Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since land farming has the potential to reduce monitoring and maintenance costs as well as clean-up liabilities, it has received much attention as a disposal alternative (Vidali, 2001)

2.3.2 Composting

Composting is a technique that involves combining contaminated soil with nonhazardous organic amendants such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting (Vidali, 2001)

2.3.3 Biopiles

Biopiles are a hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms (Sang-Hwan *et al.*, 2007).

2.3.4 Bioreactors

Slurry reactors or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment and sludge) or water through an engineered containment system (Vidali, 2001). A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants.

In general, the rate and extent of biodegradation are greater in a bioreactor system than in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pre treatment (excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (vacuum extraction) before being placed in a bioreactor (Vidali, 2001)

2.4 Microorganisms in bioremediation

Many different types of bacteria and fungi can be used for bioremediation. Microorganisms are nature's original recyclers. Their capability to transform natural and synthetic chemicals into sources of energy and raw materials for their own growth suggests that expensive chemical or physical remediation processes might be replaced with biological processes that are lower in cost and more environmentally friendly. Microorganisms therefore represent a promising, largely untapped resource for new environmental biotechnologies (Truu *et al*., 2003). Research continues to verify the bioremediation potential of microorganisms. Even dead microbial cells can be useful in bioremediation technologies. These discoveries suggest that further exploration of microbial diversity is likely to lead to the discovery of many more organisms with unique properties useful in bioremediation (Vidali, 2001). Microbes able to degrade the contaminant increase in numbers when the contaminant is present.

The use of microorganisms is not limited to one field of study of bioremediation, it has an extensive use. Oil slicks caused by oil tankers and petrol leakage into the marine environment and oil contaminated wastewater are now a constantly occurring phenomenon. A number of microorganisms can utilize oil as a source of food and many of them produce potent surface active compounds that can emulsify oil in water and facilitate its removal. Unlike chemical surfactants, the microbial emulsifier is nontoxic and biodegradable (Truu *et al*., 2003). The microorganisms capable of degrading oil include *Pseudomonas*, various *Corynebacteria*, *Mycobacteria* and some yeast. These microorganisms can be subdivided into aerobic, anaerobic, ligninolytic fungi and methylotrophs:

2.4.1 Aerobic

An aerobic organism or aerobe is an organism that has an oxygen based metabolism. Aerobes, in a process known as cellular respiration, use oxygen to oxidize substrates like fatty acid from oil in order to obtain energy. Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus* and *Mycobacterium* (Giavasis *et al*., 2006). These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds (Vidali, 2001). Many of these bacteria use the contaminant as the sole source of carbon and energy.

2.4.2 Anaerobic

An anaerobic organism or anaerobe is an organism that does not need oxygen as based metabolism. Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform (Vidali, 2001)

2.4.3 Ligninolytic fungi

Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs (Adenipekun and Fasidi, 2005).

2.4.4 Methylotrophs

The aerobic bacteria that grow by utilize methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1,2-dichloroethane (Vidali, 2001).

2.5 Environmental factors on bioremediation

Environmental variables can also greatly influence the rate and extent of biodegradation. Variables such as oxygen and nutrient availability can often be manipulated at treatment sites to enhance natural biodegradation. Other variables, such as salinity, are not usually controllable. Lack of sufficient knowledge about the effect of various environmental factors on the rate and extent of biodegradation is another source of uncertainty (Harris *et al*., 1999).

2.5.1 Oxygen

Oxygen is one of the most important requirements for microbial degradation of oil (Giavasis *et al*., 2006). However, its availability is rarely a rate limiting factor in the biodegradation of oil contaminated wastewater. Microorganisms employ oxygen incorporating enzymes to initiate attack on oil. Anaerobic degradation of certain oil which is the degradation in absence of oxygen also occurs, but usually at negligible rates. Such degradation follows different chemical paths and its ecological significance is generally considered minor. For example, studies of sediments impacted by the Amoco Cadiz spill found that, at best, anaerobic biodegradation is several orders of magnitude slower than aerobic biodegradation (Niblock, 1991).

Oxygen is generally necessary for the initial breakdown of oil, and subsequent reactions may also require direct incorporation of oxygen. Requirements can be substantial, 3 to 4 parts of dissolved oxygen are necessary to completely oxidize 1 part of oil into carbon dioxide and water. Oxygen is usually not a factor limiting the rate of biodegradation on or near the surface of the ocean, where it is plentiful and where oil can spread out to provide a large, exposed surface area. When oxygen is less available, the rates of biodegradation decrease (Niblock, 1991). Thus, oil that has sunk to the sea floor and been covered by sediment takes much longer to degrade. Oxygen availability there is determined by depth in the sediment, height of the water column and turbulence (Giavasis *et al*., 2006).

2.5.2 Nutrients

Nutrients such as nitrogen, phosphorus and iron play a much more critical role than oxygen in limiting the rate of biodegradation in marine waters. Nitrogen addition stimulated the biodegradation of alkane and polyaromatic hydrocarbons (PAHs), while phosphorus addition increased the biodegradation rate of alkane but not PAHs (Harris *et al*., 1999). Although oil is rich in the carbon required by microorganisms, it is deficient in the mineral nutrients necessary to support microbial growth. Wastewater ecosystems are often deficient in these substances because non-oil degrading microorganisms including phytoplankton consume them in competition with the oil degrading species.

Phosphorus precipitates as calcium phosphate at the high pH. Lack of nitrogen and phosphorus is most likely to limit biodegradation, but lack of iron or other trace minerals may sometimes be important (Vidali, 2001). These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon. Carbon is the most basic element of living forms and is needed in greater quantities than other elements (Vidali, 2001). Table 2.1 showed the composition of a microbial cell.

Table 2.1: Composition of a microbial cell

Element	**Percentage**
Carbon	50
Nitrogen	14
Oxygen	20
Hydrogen	8
Phosphorus	3
Sulfur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chloride	0.5
Iron	0.2
All others	0.3

2.5.3 Temperature

At low temperature, the rate of oil metabolism by microorganisms decreases. So, lighter fractions of petroleum which is palm oil become less volatile, thereby leaving the oil constituents that are toxic to microbes in the water for a longer time and depressing microbial activity (Phillips *et al.*, 1974). The rates of biodegradation are faster at higher temperature (Thomas, 1987). The diffusion coefficients and the solubility of lipids in aqueous media increase significantly with rising temperature. Under thermopile conditions, lipids become more accessible to microorganisms (Chipasa *et al.,* 2006). A temperature increase affects a decrease in viscosity, thereby affecting the degree of distribution and increasing diffusion rates of organic compounds. Therefore, higher reaction rates due to smaller boundary layers are